

TOXICITY TEST REPORT

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INTRODUCTION

The North Fork of Clear Creek (NFCC) is a site located in the Central Region of Colorado, west of Denver. It is in the Rocky Mountain region and serves as one of the main tributaries to Clear Creek, part of the South Platte River drainage basin. Concerns over the site are not completely known, but the sources of impacts are mines, both abandoned and operating, with the resulting heavy metals and low pH impacts. The purpose behind this study is to establish a baseline for the extent of contamination (toxicity) for both the water column and the sediment at selected sites throughout the stream reach and tributaries. This study should also aid in tracking the trends (changes) in the watershed during the study. Seven of the water column samples were tested using the *Daphnia magna* four day survival and growth toxicity test method. The remaining two samples were tested using a *C. dubia* 48 hour acute toxicity test method. The sediment samples were analyzed with a 10-day sediment toxicity test using *Hyalella azteca*, as well as a 4-day sediment toxicity test using *D. magna*. The *D. magna* method is experimental, as is the *P. promelas* 8-day sediment embryo-larval test that was also used. Background data does exist for this site, due to testing of various segments of the stream for the Superfund Program.

METHODS and MATERIALS

Water column and sediment toxicity samples were collected at NFCC by USEPA field personnel and shipped to SBI at the Newtown laboratory. These samples were collected over 9-20-97 and 9-22-97 and were received at the laboratory 9-23-97. Arrival chemistries were determined for all samples on receipt, Table 1.

The tests with the water column samples were started 9-23-97. Two water column samples, SW-05 and SW-07A, were analyzed using an acute *C. dubia* test procedure. These samples were collected as part of a separate study into the remediation of the Argo Tunnel, Idaho Springs, CO. The acute test method used a 48 hour test duration, with daily test solution renewal, Table 10. Test solution volume was 20 mls and the test used <24 hour old *C. dubia* at the start.

The remaining 7 samples were analyzed using the *D. magna* 4-day survival and growth test method. The *D. magna* tests, Table 7, used a four day test duration, with a daily test solution renewal. They were fed daily 0.3 ml of *Selenastrum capricornutum* algae (80-100 million cells/ml) and 0.2 ml blended alfalfa extract (7.5 g alfalfa/1000 ml deionized water). The test solution volume

was 50 ml. The tests were started using <24 hour old *D. magna*, in a 12-hour age range. A complete listing of this procedure is contained in Table 7.

The sediment toxicity testing procedures used the standard 10-day *H. azteca* survival and growth sediment test method, Table 8. This was a static-renewed test and used 7 day old *H. azteca*. The water was renewed and the test animals were fed daily with 2 ml of a mixture of a 1:1 suspension of *S. capricornutum* (80-100 million cells/ml) and alfalfa extract (5 g alfalfa/500 ml deionized water). In addition to the *H. azteca*, *D. magna* were also exposed in this procedure, Table 7. This was done by adding five *D. magna* to each test beaker, so that the two species were exposed concurrently. The *D. magna* underwent the same procedures as the *H. azteca*, for four days. At the end of the four day duration, the *D. magna* were pipetted from the test containers, for final survival and dry weights.

The final sediment toxicity testing procedure was a 7-day *P. promelas* embryo-larval test method, Table 9, currently under development. This method involved exposing the fish embryo directly on the surface of the sediment, allowing for interaction between the test animal and the sediment. This method uses 40 ml of sediment and 60 ml of overlying water. The water is replaced daily and the live/dead/hatched embryo and larvae are counted.

The water column tests used moderately hard reconstituted water (MHRW), with selenium added, as the standard control and dilution water. The sediment toxicity tests used reformulated moderately hard reconstituted water (RMHRW) as the overlying water. Both were prepared using a Millipore Super-Q® deionized water system. The control sediment was a top soil control (TS Control) prepared by grinding commercial top soil in a blender and then sieving the material through a # 8 sieve (2.36 mm) to remove the debris. This material was stored dry, and wetted using RMHRW (100 ml sediment/45 ml water).

The data were analyzed using several methods. The LC50 values were determined using Trimmed Spearman-Kärber, v1.5. The NOEC and LOEC levels, as well as all t-tests, were determined using Sigma Stat®. All ANOVA and t-test alpha levels were 0.05. All growth determinations are based on mean dry weight.

RESULTS and DISCUSSION

Results from both the water column and sediment toxicity tests indicated both acute and chronic toxicity at the test sites. The results from SW49B indicates it is acceptable as an upstream reference site, for both water column and sediment toxicity testing (with *H. azteca*).

Daphnia magna Water Column Toxicity Tests

Tests with the water column sample using the *D. magna* 4-day test method started 9-23 ran

into a problem with control survival. We do not know what caused this problem, but the controls for all tests died on day 2. This resulted in the need to restart all tests with new test animals. These tests, which included samples SW36, SW37, SW39, and SW43, were restarted 9-28-97. A modified definitive dilution series was used, 12.5% to 100%. The second set of tests did meet the control survival criteria, as well as the growth criteria. The initial weight of the animals used to start these tests was 8.7 μg , so the 10X the initial weight criteria was 87 μg . The remaining tests, with SW40, SW48 and SW49B, were started 9-24-97. SW40 and SW48 used a dilution series of 6.25% to 100%. SW49B was analyzed as a 100% only, profile sample. The *D. magna* used to start these tests weighed 7.5 μg , so the 10X the initial weight control growth criteria for these tests is 75 μg . All results are contained in Tables 2 and 3.

For sample SW36, control survival was 100% and growth in the control was 106 μg . The survival NOEC value for this sample was 25%, with an LC50 value of 46.6%. The growth NOEC value was 12.5% and the IC25 value was 16.7%

For sample SW37, control survival was 95% and growth in the control was 90 μg . The survival NOEC value was 25% and the LC50 value was 47.3%. The growth NOEC value was 25% and the IC25 was 28.1%

For sample SW39, control survival was 95%, with a control growth of 98.25 μg . The survival NOEC value was 25%, with an LC50 value of 52.8%. The growth NOEC value was 12.5% and the IC25 value was 20.2%.

For sample SW40, control survival was 100%, with a control growth of 128 μg . The survival NOEC value was 25% and the LC50 value was 51.8%. The growth NOEC value was <6.25%, with an IC25 value of 15.8%.

For sample SW43, control survival was 100%, with a control growth of 92.5 μg . The survival NOEC value was 25%, with an LC50 value of 49.1%. The growth NOEC value was 12.5%, with an IC25 value of 16.1%.

For sample SW48, control survival was 100%, with a control growth of 90 μg . The survival NOEC value was 50%, with an LC50 value of 65.1%. The growth NOEC value was 12.5% and the IC25 value was 16.1%.

For the SW49B profile sample, the control survival was 100%, with a growth of 90 μg . Survival in the SW49B 100% sample was 100% and the growth of the *D. magna* in the sample was 146 μg .

In summary, the samples collected from SW36, SW37, SW39, SW40, SW43 and SW48 showed both acute and chronic toxicity in the *D. magna* 4-day survival and growth test. At a minimum, the 100% sample was acutely toxic, with the 50% sample being acutely toxic in five of these six samples. All samples also showed a chronic toxicity, with five of the six growth NOEC

values being 12.5% or less. SW49B was not toxic, with the survival being equal to that of the control, while the growth of the *D. magna* in the sample exceeded the growth in the control by over 35%. SW49B is acceptable as an upstream reference site. The data indicates the stream is picking up toxicity somewhere above SW48, but below SW49B. As the sampling stations move downstream, the toxicity remains.

Hyalella azteca Sediment Toxicity Tests

The survival of the control animals (68.75%) in this test was below the minimum acceptability criteria of 80%. The results from the upstream reference site, SW49B will be used for all data analysis. Results from sediment toxicity testing with *H. azteca* indicate only one site is acutely toxic, SW36 (survival = 51.25%). Analyzing this data against that of the upstream sample, SW49B (survival = 98.75%) with a t-test ($\alpha = 0.05$) resulted in a pvalue of 0.0002, which indicates the survival in this sample is statistically different from that of the control. For growth, four of the samples (SW07A, SW36, SW39, and SW40) were determined to have growth statistically different from that of SW49B (Table 4).

Daphnia magna Sediment Toxicity Tests

None of the samples were determined to be toxic based on the results of the *D. magna* sediment toxicity tests. Survival in the control was 85%, with a growth of 177.7 μg . The lowest sample survivals were 55% (SW43) and 60% (SW37). Neither was determined to be statistically different from the control based on a t-test, $\alpha = 0.05$ (Table 5). The same was true for the growth endpoint, the growth in six of the nine samples exceeding the control growth. Analysis of the remaining three found none of the three were statistically different from the control growth. The lowest growth was in SW43, 139.3 μg (Table 5). The pvalue for this sample, when compared to the control, was 0.3633. NOTE: The survival of the *D. magna* in the control sediment is below the 90% control survival criteria used in the water column test. Since the use of the *D. magna* 4-day test procedure with sediments is new, insufficient data currently exists to determine a control survival criteria for the sediment test method. It is possible that for this method, a control survival criteria of 80% is appropriate. Since we are not certain at this point, it is best interpret these results with caution.

P. promelas Sediment Toxicity Tests

No toxicity was found in the sediment samples tested using the FHM embryo-larval sediment test method. Survival in the control sample was 60%, below the minimum survival criteria of 80%. The best survival was in SW36 (90%), so this was used as the comparison for estimating toxic sediments. Six of the nine sediments exceeded 75% survival, and were determined to not be impacted. These six were the lowest downstream samples on NFCC, SW36, SW37, SW39, SW40, and SW42. The two upstream samples, SW-48 and SW49B, had the lowest overall survival on NFCC, 67.5% and 72.5% respectively. Lowest survival for any site tested was SW05, with a survival of 40%. The variability in the sample was excessive, so it was not determined to be statistically

different from the control. The pvalue was 0.599, on the border of being significantly different.

In summary, the sediment toxicity data indicate the only sediment on NFCC acutely toxic to the *H. azteca* was SW36, the station farthest downstream. For growth, SW39 and SW40 were determined to be different from the upstream control, SW49B. The *D. magna* and *P. promelas* sediment tests did not detect toxicity in any of the samples.

Argo Tunnel Toxicity Testing

The toxicity of the two stations from mainstem of Clear Creek indicate the stream is not acutely toxic upstream at SW07A, *C. dubia* LC50 >100%. At the next downstream station, acute toxicity increases significantly, with the *C. dubia*, LC50 = 19.8%. Neither sediment sample was acutely toxic to the *H. azteca*, with survivals of 96.25% in SW05 and 80% in SW07A. Growth in SW05 was also acceptable, 68 μ g, while the growth in SW07A was determined to be statistically different from the SW49B control, 52 μ g versus 83 μ g. No toxicity was detected using the *D. magna* sediment toxicity testing method. For the FHM embryo-larval sediment test, the survival in SW05 was 40%, but the pvalue was 0.0599, above the 0.05 significance level. However, examining the coefficient of variance for this test indicates excessive variability. So, while the data analysis indicates no difference from the control, a survival of 40% does indicate the potential for some contaminate, since the survival in the upstream sample, SW07A, was 75%. This sample (SW05) could possibly be toxic to the FHM embryo, but more data is required for a definitive answer. As it stands, the water column is acutely toxic to the *C. dubia*, while the sediments may be toxic to the FHM embryo.

Table 1. Arrival Chemistries

	DATE	TEMP	pH	ALK	HARD	COND	D.O.
SAMPLE	RECEIVED	(°C)	(S.U.)	(PPM)	(PPM)	(S/cm)	(PPM)
SW05	9/23/97	1.4	7.24	26	68	150	9.7
SW07A	9/23/97	1.3	7.25	30	60	132	9.9
SW36	9/23/97	1.1	6.93	12	148	347	9.4
SW37	9/23/97	1.4	6.68	8	139	317	10.4
SW39	9/23/97	1.2	6.40	9	151	345	10.2
SW40	9/23/97	1.3	6.24	8	153	334	9.1
SW43	9/23/97	1.1	6.20	10	132	300	9.9
SW48	9/23/97	1.1	7.14	24	48	109	9.6
SW49B	9/23/97	1.2	7.24	26	68	150	9.9
MHW	9/21/98	23.2	7.94	62	94	331	8.2

MHW Moderately Hard Reconstituted Water-Control for Water Column Tests

Table 2. *D. magna* Survival and Weight Data, *C. dubia* survival data
Water Column Toxicity Tests.

SAMPLE ID	CONC. %	SURV. %	C.V. %	Wt. (ug)	C.V. %
SW05	control	95	10.5	N/A	N/A
<i>C. dubia</i>	3.125	100	0	N/A	N/A
acute	6.25	100	0	N/A	N/A
	12.5	95	10.5	N/A	N/A
	25	20	115.5	N/A	N/A
	50	10	200	N/A	N/A
SW07A	control	100	0	N/A	N/A
<i>C. dubia</i>	6.25	100	0	N/A	N/A
acute	12.5	100	0	N/A	N/A
	25	100	0	N/A	N/A
	50	100	0	N/A	N/A
	100	90	12.8	N/A	N/A
SW36	control	100	0	106	13.9
	12.5	100	0	90.5	3.3
	25	100	0	57.5	13.1
	50	40	91.3	19	96.3
	100	0	0	0	0
SW37	control	95	10.5	90	12.1
	12.5	95	10.5	79.5	3.2
	25	95	10.5	74.6	11.2
	50	40	70.7	17	75.9
	100	0	0	0	0
SW39	control	95	10.5	98.25	14.1
	12.5	95	10.5	93.5	9
	25	85	11.8	61.5	17.9
	50	65	29.5	27.5	39.6
	100	0	0	0	0

Table 2. *D. magna* Survival and Weight Data, Water Column Toxicity Tests

SAMPLE ID	CONC. %	SURV. %	C.V. %	Wt. (ug)	C.V. %
SW40	control	100	0	128	5.8
	6.25	100	0	99.5	17.6
	12.5	100	0	101	15
	25	100	0	84	24.3
	50	55	34.8	40	33.9
	100	0	0	0	0
SW43	control	100	0	92.5	11.9
	12.5	95	10.5	95.5	11.8
	25	85	11.8	65.5	25.2
	50	60	38.5	12.3	92.4
	100	0	0	0	0
SW48	control	100	0	90	12.4
	6.25	95	10.5	92.5	11.2
	12.5	100	0	80.5	9.4
	25	95	10.5	39	39.4
	50	90	22.2	34.5	26.5
	100	0	0	0	0
SW49B	control	100	0	90	12.4
	100	100	0	146	13.5

Table 3. *D. magna* and *C. dubia* NOEC, LOEC, LC50 and IC25 Values, Survival and Growth.

		Survival (%)			Growth(%)	
Sample	NOEC	LOEC	LC50	NOEC	LOEC	IC 25
SW05	12.5	25	19.8	N/A	N/A	N/A
SW07A	>100	>100	>100	N/A	N/A	N/A
SW36	25	50	46.6	12.5	25	16.7
SW37	25	50	47.3	25	>25	28.1
SW39	25	50	52.8	12.5	25	20.2
SW40	25	50	51.8	<6.25	<6.25	15.8
SW43	25	50	49.1	12.5	25	22.8
SW48	50	100	65.1	12.5	50	16.1
SW49B*	>100	>100	>100	>100	>100	>100

Table 4. Survival and Growth Data for *H. azteca* Sediment Toxicity Tests.

Sample	Survival %	C.V.%	p value	Wt. ug	C.V.%	p value
TS CONT	68.75	15	N/A	67	19.2	N/A
SW05	96.25	5	0.4476	68	18	0.2178
SW07A	80	24.5	0.0878	52	31.7	0.0483
SW36	51.25	25.7	0.0002	58	9.4	0.0381
SW37	95	4.3	0.1733	66	29.9	0.2491
SW39	91.25	15.7	0.3654	51	7.5	0.0151
SW40	78.75	19	0.0551	46	22.5	0.0125
SW43	95	4.3	0.1733	61	6.2	0.0583
SW48	93.75	8	0.3401	61	12.3	0.0671
SW49B*	98.75	2.5	N/A	83	22.2	N/A

*Upstream reference site used for data analysis with t-tests.

Table 5. Survival and Growth data for *D. magna* Sediment Toxicity Test.

Sample	Survival %	C.V.%	p value	Wt. μ g	C.V.%	p value
TS CONT	85	22.4	N/A	177.7	24.6	N/A
SW05	85	11.8	0.7797	215.8	14.2	0.2045
SW07A	80	28.8	0.8398	211	14.6	0.2591
SW36	100	0	0.1457	171.5	8.5	0.7954
SW37	60	38.3	0.1153	211.5	20.5	0.3148
SW39	100	0	0.1457	196.5	9.1	0.4579
SW40	85	22.4	1	162.8	18.4	0.5919
SW43	55	63.6	0.2419	139.3	46.6	0.3633
SW48	90	22.2	0.6496	197.8	21.4	0.5359
SW49B	80	20	0.6237	188	6.7	0.6684

Table 6. Survival Data for *P. promelas* Sediment Toxicity Test.

Sample	Survival %	C.V.%	p value*
MTSCont.	60	13.6	N/A
SW05	40	102.1	0.0599
SW07A	75	7.7	N/A
SW36	90	15.7	N/A
SW37	75	23.1	N/A
SW39	87.5	14.4	N/A
SW40	80	10.2	N/A
SW43	87.5	10.9	N/A
SW48	67.5	22.2	0.0718
SW49B	72.5	13.2	N/A

* Comparisons made using SW36 (sample with best survival) in a t-test.

TABLE 7. Standard Operating Procedures for *Daphnia magna* Survival and Growth Toxicity Tests for Superfund Samples.

<u>TEST PARAMETER</u>	<u>CONDITION</u>
Test Type	static-renewal
Test Duration	4 days
Temperature	25 °C (± 1 °C)
Photoperiod	16 h light: 8 h dark
Test Chamber Size	60 ml
Test Solution Volume	50 ml
Renewal of Test Solution	daily
Age of Test Organisms	< 24 hrs old
No. Organisms/Test Chamber	5
No. Replicate Test Chambers	4
No. Organisms/concentration	20
Feeding Regime	0.3 ml algae & 0.2 ml alfalfa
Test Solution Aeration	None
Dilution Water	Moderately Hard Water + Selenium
Endpoint	Survival and Mean Dry Weight
Test Acceptability	90% or greater control survival control growth 10X initial weight

TABLE 8. Standard Operating Procedures for *Hyalella azteca* acute toxicity tests for Superfund samples. (Also for multi species test with *Daphnia magna*)

<u>TEST CRITERIA</u>	<u>SPECIFICATIONS</u>
Test Type	Static-renewal
Test Duration	10 days
Temperature	23 °C ± 1 °C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	200 ml
Sediment Volume	75 ml
Overlying Water Volume	125 ml
Renewal of Test solution	daily
Age of Test Organisms	7-days old, 24 hour age range
Number of Organisms/ per test chamber	20 (5 <24 hr old <i>D. magna</i>)
Number of Replicate Chambers/Conc.	4 (Same for <i>D. magna</i>)
Number of Organisms/ Concentration	80 (20 <i>D. magna</i>)
Feeding	2 ml algae/alfalfa
Dilution Water	Reformulated Moderately Hard Reconstituted Water
Control Sediment	Potting Soil
Endpoint	Mortality, difference from control Growth, difference from control
Test Acceptability	> 80% survival in the controls

D. magna, > 90% and final weight 10X initial

TABLE 9. Standard Operating Procedures for *Pimephales promelas* embryo-larval sediment toxicity test.

<u>TEST CRITERIA</u>	<u>SPECIFICATIONS</u>
Test Type	Static-renewal
Test Duration	7 days
Temperature	25 °C ± 1 °C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	100 ml
Sediment Volume	25 ml
Overlying Water Volume	75 ml
Renewal of Test solution	daily
Age of Test Organisms	24-48 hour old embryo
Number of Organisms/ per test chamber	10
Number of Replicate Chambers/Conc.	4
Number of Organisms/ Concentration	40
Feeding	none
Dilution Water	Reformulated Moderately Hard Reconstituted Water
Control Sediment	modified top soil
Endpoints	Mortality, difference from control Hatch ability and abnormal embryo (terata)

Test Acceptability

> 80% survival in the controls

TABLE 10. Standard Operating Procedures for *Ceriodaphnia dubia* acute toxicity tests for Superfund Samples

<u>TEST CRITERIA</u>	<u>SPECIFICATIONS</u>
Test Type	Static-renewal
Test Duration	2 days
Temperature	20 °C ± 1 °C
Photoperiod	16 hr light/8 hr dark
Test Chamber Size	30 ml
Test Solution Volume	15 ml
Renewal of Test solution	daily
Age of Test Organisms	<24 hours old
Number of Organisms/ per test chamber	5
Number of Replicate Chambers/Conc.	4
Number of Organisms/ Concentration	20
Feeding	Fed while holding, prior to start of test.
Dilution Water	Moderately Hard Reconstituted Water
Endpoints	Survival, LC50
Test Acceptability	> 90% survival in the controls